



INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification ⁷ : A61F 13/00	A1	(11) International Publication Number: WO 00/23023 (43) International Publication Date: 27 April 2000 (27.04.00)
(21) International Application Number: PCT/US99/24546 (22) International Filing Date: 20 October 1999 (20.10.99) (30) Priority Data: 60/104,999 20 October 1998 (20.10.98) US (71) Applicant: THE UNIVERSITY OF NORTH CAROLINA AT CHAPEL HILL [US/US]; Campus Bos 4105, 308 Bynum Hall, Chapel Hill, NC 27599-4105 (US). (72) Inventor: BOUCHER, Richard, C., Jr.; 735 Gimghoul Road, Chapel Hill, NC 27514 (US). (74) Agents: SIBLEY, Kenneth, D. et al.; Myers, Bigel, Sibley, & Savoie, P.A., P.O. Box 37428, Raleigh, NC 27627 (US).		(81) Designated States: AU, CA, JP, European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE). Published <i>With international search report. Before the expiration of the time limit for amending the claims and to be republished in the event of the receipt of amendments.</i>
(54) Title: METHODS OF HYDRATING MUCOSAL SURFACES (57) Abstract A method of hydrating nasal airway surfaces in a subject in need of such treatment comprises topically applying a sodium channel blocker to a nasal airway surface of the subject in an amount effective to inhibit the reabsorption of water by the surface. The channel blocker may be a pyrazinolyguanidine sodium channel blocker, such as benzamil, phenamil, amiloride, or pharmaceutically acceptable salts thereof. The method may further comprise the step of topically applying a P2Y ₂ receptor agonist to a nasal airway surface of the subject in an amount effective to stimulate chloride secretion by the nasal airway surface. In a preferred embodiment, the sodium channel blocker is a covalent conjugate of a pyrazinoylguanidine sodium channel blocker and a non-absorbable carrier moiety (e.g., albumin, polyethylene glycol). Such compounds may also be administered to other mucosal surfaces where it is desired to inhibit the reabsorption of water.		

FOR THE PURPOSES OF INFORMATION ONLY

Codes used to identify States party to the PCT on the front pages of pamphlets publishing international applications under the PCT.

AL	Albania	ES	Spain	LS	Lesotho	SI	Slovenia
AM	Armenia	FI	Finland	LT	Lithuania	SK	Slovakia
AT	Austria	FR	France	LU	Luxembourg	SN	Senegal
AU	Australia	GA	Gabon	LV	Latvia	SZ	Swaziland
AZ	Azerbaijan	GB	United Kingdom	MC	Monaco	TD	Chad
BA	Bosnia and Herzegovina	GE	Georgia	MD	Republic of Moldova	TG	Togo
BB	Barbados	GH	Ghana	MG	Madagascar	TJ	Tajikistan
BE	Belgium	GN	Guinea	MK	The former Yugoslav Republic of Macedonia	TM	Turkmenistan
BF	Burkina Faso	GR	Greece			TR	Turkey
BG	Bulgaria	HU	Hungary	ML	Mali	TT	Trinidad and Tobago
BJ	Benin	IE	Ireland	MN	Mongolia	UA	Ukraine
BR	Brazil	IL	Israel	MR	Mauritania	UG	Uganda
BY	Belarus	IS	Iceland	MW	Malawi	US	United States of America
CA	Canada	IT	Italy	MX	Mexico	UZ	Uzbekistan
CF	Central African Republic	JP	Japan	NE	Niger	VN	Viet Nam
CG	Congo	KE	Kenya	NL	Netherlands	YU	Yugoslavia
CH	Switzerland	KG	Kyrgyzstan	NO	Norway	ZW	Zimbabwe
CI	Côte d'Ivoire	KP	Democratic People's Republic of Korea	NZ	New Zealand		
CM	Cameroon			PL	Poland		
CN	China	KR	Republic of Korea	PT	Portugal		
CU	Cuba	KZ	Kazakstan	RO	Romania		
CZ	Czech Republic	LC	Saint Lucia	RU	Russian Federation		
DE	Germany	LI	Liechtenstein	SD	Sudan		
DK	Denmark	LK	Sri Lanka	SE	Sweden		
EE	Estonia	LR	Liberia	SG	Singapore		

5

METHODS OF HYDRATING MUCOSAL SURFACES

Related Applications

10 This application claims priority from provisional application serial number 60/104,999, filed October 20, 1998, the disclosure of which is incorporated by reference herein in its entirety.

Field of the Invention

15 The present invention concerns methods of hydrating nasal airway surfaces in patients in need of such treatment, along with compounds and compositions useful for carrying out such methods.

Background of the Invention

20 The current therapy for hydrating nasal airway surfaces is to apply normal saline via drops or spray delivery devices. The disadvantage of this simple approach is that the duration of effect is short. It would be desirable to provide a way to achieve a more lasting hydration of nasal airway surfaces.

25 U.S. Patent No. 5,789,391 to Jacobus describes methods of treating sinusitis with uridine triphosphates and related compounds such as UTP or P¹,P⁴-di(uridine-5' tetraphosphate (U₂P₄) in an amount effective to promote drainage of congested fluid in the sinuses by hydrating mucous secretions or by stimulating ciliary beat frequency in the sinuses.

30 U.S. Patent No. 4,501,729 to Boucher describes the use of respirable or non-respirable amiloride to hydrate airway mucous secretions, and U.S. Patent No. 5,656,256 to Boucher and Stutts describes the use of respirable or non-respirable benzamil and phenamil to hydrate lung mucous secretions. The use of amiloride,

benzamil or phenamil to hydrate nasal airway surfaces is neither disclosed nor suggested.

Summary of the Invention

5 A first aspect of the present invention is a method of hydrating nasal airway surfaces in a subject in need of such treatment. The method comprises topically applying a sodium channel blocker to a nasal airway surface of the subject in an amount effective to inhibit the reabsorption of water by the nasal airway surface. The channel blocker may be a pyrazinoylguanidine sodium channel blocker, such as
10 benzamil, phenamil, amiloride, or a pharmaceutically acceptable salt thereof.

 The method may further comprise the step of topically applying a P2Y₂ receptor agonist to a nasal airway surface of the subject in an amount effective to stimulate chloride secretion, and thereby stimulate water secretion, by the nasal airway surface.

15 A second aspect of the present invention is a method of hydrating mucosal surfaces such as airway surfaces in a subject in need of such treatment, comprising topically applying a sodium channel blocker to a mucosal surface such as an airway surface of the subject in an amount effective to inhibit the reabsorption of water by the surface, wherein the sodium channel blocker is a covalent conjugate of a
20 pyrazinoylguanidine sodium channel blocker and a non-absorbable carrier moiety (e.g., polyethylene glycol, albumin; carbohydrate).

 Again, the method may further comprise the step of topically applying a P2Y₂ receptor agonist to a mucosal surface such as an airway surface of the subject in an amount effective to stimulate chloride secretion by the surface.

25 A third aspect of the present invention is a pharmaceutical formulation, comprising a sodium channel blocker in a pharmaceutically acceptable carrier (e.g., an aqueous carrier solution); wherein the sodium channel blocker is a covalent conjugate of a pyrazinoylguanidine sodium channel blocker and a non-absorbable carrier moiety; preferably subject to the proviso that the non-absorbable carrier
30 moiety is not bovine serum albumin or rabbit serum albumin. The composition may further contain a P2Y₂ receptor agonist.

 A fourth aspect of the present invention is a compound useful as a sodium channel blocker, the compound comprising a covalent conjugate of a

pyrazinoylguanidine sodium channel blocker and a non-absorbable carrier moiety; preferably subject to the proviso that the non-absorbable carrier moiety is not bovine serum albumin, rabbit serum albumin or agarose. For example, the carrier moiety can be polyethylene glycol or human serum albumin, or a carbohydrate.

5 A fifth aspect of the present invention is the use of compounds as described above for the preparation of a medicament for the treatment of disorders as described herein.

The present invention is explained in greater detail in the specification below.

10 Detailed Description of Preferred Embodiments

The term "airway surface" as used refers to airway surfaces below the larynx and in the lungs, as well as air passages in the head, including the sinuses, in the region above the larynx.

15 The term "nasal airway surface" as used herein refers to the airways and associated air passages in the head, including the sinuses, in the region above the larynx. This definition excludes airways below the larynx, which have been the target of drug delivery for different purposes.

20 The term alkyl or loweralkyl as used herein refers to C1 to C4 alkyl, which may be linear or branched and saturated or unsaturated. Cycloalkyl is specified as such herein, and is typically C3, C4 or C5 to C6 or C8 cycloalkyl. Alkenyl or loweralkenyl as used herein likewise refers to C1 to C4 alkenyl, and alkoxy or loweralkoxy as used herein likewise refers to C1 to C4 alkoxy. Halo as used herein refers to any halogen, such as chloro, fluoro, bromo, or iodo.

25 The term "non-absorbable carrier moiety" as used herein refers to a functional group that serves to trap the compound containing the group on airway surfaces, or adhere the compound to airway surfaces, and partly or wholly inhibits the absorption of the compound by the surface on which it is deposited.

30 Subjects that may be treated by the methods of the present invention include patients afflicted with cystic fibrosis, primary ciliary dyskinesia, chronic bronchitis, chronic obstructive airway disease, artificially ventilated patients, patients with acute pneumonia, etc. The present invention may be used to obtain a sputum sample from a patient by administering the active compounds to at least one lung of a patient, and then inducing or collecting a sputum sample from that patient.

Subjects that may be treated by the method of the present invention also include patients being nasally administered supplemental oxygen (which tends to dry the airway surfaces), patients afflicted with an allergic disease or response (*e.g.*, an allergic response to pollen, dust, animal hair or particles, insects or insect particles, etc.) that affects nasal airway surfaces, patients afflicted with a bacterial infection (*e.g.*, staphylococcus infections such as staphylococcus aureus infections, Hemophilus influenza infections, streptococcus pneumoniae infections, pseudomonas infections, etc.) of the nasal airway surfaces, an inflammatory disease that affects nasal airway surfaces, or patients afflicted with sinusitis (wherein the active agent or agents are administered to promote drainage of congested mucous secretions in the sinuses by administering an amount effective to promote drainage of congested fluid in the sinuses).

The present invention may be used to hydrate mucosal surfaces other than airway surfaces. Such other mucosal surfaces include gastrointestinal surfaces, oral surfaces, genito-ureteral surfaces, ocular surfaces or surfaces of the eye, the inner ear, and the middle ear. For example, the active compounds of the present invention may be administered by any suitable means, including orally or rectally, in an amount effective to combat constipation in a subject.

The present invention is concerned primarily with the treatment of human subjects, but may also be employed for the treatment of other mammalian subjects, such as dogs and cats, for veterinary purposes.

1. Active agents.

Pyrazinoylguanidine sodium channel blockers are disclosed in U.S. Patent No. 3,313,813 to Cragoe. (Applicant specifically intends that the disclosure of this and all other U.S. Patent references cited herein be incorporated by reference herein in their entirety).

Amiloride, one particular pyrazinoylguanidine sodium channel blocker, is described at Merck Index Registry No. 426 (12th Ed. 1996).

Benzamil (also known as 3,5-diamino-6-chloro-N-(benzylaminoaminomethylene) pyrazinecarboxamide) and phenamil (also known as 3,5-diamino-6-chloro-N-(phenylaminoaminomethylene)pyrazinecarboxamide) are known compounds and are disclosed in U.S. Pat. No. 3,313,813 to E. Cragoe

(applicant specifically intends that the disclosure of this and all other patents cited herein be incorporated herein by reference).

Various additional pyrazinoylguanidine sodium channel blockers that are amiloride analogs are disclosed and described in T. Kleyman and E. Cragoe,
5 *Amiloride and its Analogs as Tools in the Study of Ion Transport*, J. Membrane Biol. 105, 1-21 (1988).

Specific examples of active compounds that may be used to carry out the present invention are the pyrazinoylguanidine sodium channel blockers disclosed in U.S. Pat. No. 3,313,813, incorporated by reference above. Such compounds have the
10 formula:

wherein:

X is selected from the group consisting of chloro, bromo, iodo, loweralkyl, lower-cycloalkyl having from 3 to 7 carbons, phenyl, chlorophenyl, bromophenyl, X'-
15 thio and X'-sulfonyl wherein X' is selected from the group consisting of loweralkyl and phenyl-loweralkyl. Preferably, X is chloro.

Y is selected from the group consisting of hydroxyl, mercapto, loweralkyloxy, loweralkylthio, chloro, loweralkyl, lowercycloalkyl having from 3 to 6 carbons, phenyl, amino having the structure:
20

wherein:

R is selected from the group consisting of hydrogen, amino, amidino, lower-cycloalkyl having 3 to 6 carbon atoms, loweralkyl, hydroxyloweralkyl, halo-loweralkyl, lower-(cycloalkylalkyl) having 3 to 6 carbons in the ring, phenyl-
25 loweralkyl, lower-(alkylaminoalkyl), lower-alkenyl, phenyl, halophenyl, and lower-alkylphenyl. In one preferred embodiment, Y is chloro; in another preferred embodiment, Y is amino.

R₁ is selected from the group consisting of hydrogen, loweralkyl, loweralkenyl, and additionally;

30 R and R₁ can be joined to form a lower alkylene.

R₂ is selected from the group consisting of hydrogen and loweralkyl. Preferably, R, R₁, and R₂ are hydrogen.

R_3 and R_4 respectively are independently selected from the group consisting of hydrogen, loweralkyl, hydroxy-loweralkyl, phenyl-loweralkyl, (halophenyl)-loweralkyl, lower-(alkylphenylalkyl), (loweralkoxyphenyl)-loweralkyl, naphthyl-loweralkyl, (octahydro-1-azocinyl)-loweralkyl, pyridyl-loweralkyl, and loweralkyl radicals linked to produce with the nitrogen atom to which they are attached a 1-pyrrolidinyl, piperidino, morpholino, and a 4-loweralkyl-piperazinyl group, and phenyl. Preferably, R_3 is hydrogen, phenyl, or phenylalkyl. Preferably, R_4 is hydrogen.

2. Pharmaceutically acceptable salts.

The term "active agent" as used herein, includes the pharmaceutically acceptable salts of the compound, such as (but not limited to) benzamil hydrochloride or phenamil hydrochloride. Pharmaceutically acceptable salts are salts that retain the desired biological activity of the parent compound and do not impart undesired toxicological effects. Examples of such salts are (a) acid addition salts formed with inorganic acids, for example hydrochloric acid, hydrobromic acid, sulfuric acid, phosphoric acid, nitric acid and the like; and salts formed with organic acids such as, for example, acetic acid, oxalic acid, tartaric acid, succinic acid, maleic acid, fumaric acid, gluconic acid, citric acid, malic acid, ascorbic acid, benzoic acid, tannic acid, palmitic acid, alginic acid, polyglutamic acid, naphthalenesulfonic acid, methanesulfonic acid, p-toluenesulfonic acid, naphthalenedisulfonic acid, polygalacturonic acid, and the like; and (b) salts formed from elemental anions such as chlorine, bromine, and iodine.

Active agent used to prepare compositions for the present invention may alternatively be in the form of a pharmaceutically acceptable free base of active agent. Because the free base of the compound is less soluble than the salt, free base compositions are employed to provide more sustained release of active agent to the lungs. Active agent present in the lungs in particulate form which has not gone into solution is not available to induce a physiological response, but serves as a depot of bioavailable drug which gradually goes into solution.

3. Formulations and administration.

A third aspect of the present invention is a pharmaceutical formulation, comprising a sodium channel blocker in a pharmaceutically acceptable carrier (e.g., an aqueous carrier solution); wherein the sodium channel blocker is a covalent conjugate of a pyrazinoylguanidine sodium channel blocker and a non-absorbable carrier moiety; preferably subject to the proviso that the non-absorbable carrier moiety is not bovine serum albumin or rabbit serum albumin. In general, the sodium channel blocker is included in the composition in an amount effective to inhibit the reabsorption of water by airway surfaces, particularly nasal airway surfaces. As discussed below, the composition may further comprise a P2Y₂ receptor agonist or a pharmaceutically acceptable salt thereof (also sometimes referred to as an "active agent" herein). The P2Y₂ receptor agonist is typically included in an amount effective to stimulate chloride and water secretion by airway surfaces, particularly nasal airway surfaces.

The active compounds disclosed herein may be administered to the nasal airway surfaces of a patient by any suitable means, including as a spray, mist, or droplets of the active compounds in a pharmaceutically acceptable carrier such as physiological saline solution or distilled water. For example, the active compounds may be prepared as formulations and administered as described in U.S. Patent No. 5,789,391 to Jacobus, the disclosure of which is incorporated by reference herein in its entirety.

In one preferred embodiment they are administered by administering an aerosol suspension of respirable or non-respirable particles (preferably non-respirable particles) comprised of the active compound, which the subject inhales through the nose. The respirable or non-respirable particles may be liquid or solid. The quantity of active agent included may be an amount sufficient to achieve dissolved concentrations of active agent on the nasal airway surfaces of the subject of from about 10⁻⁷ to about 10⁻³ Moles/liter, and more preferably from about 10⁻⁶ to about 10⁻⁴ Moles/liter.

In one embodiment of the invention, the particulate active agent composition may contain both a free base of active agent and a pharmaceutically acceptable salt such as benзамил hydrochloride or phenamil hydrochloride to provide both early release of and sustained release of active agent for dissolution into the mucous secretions of the nose. Such a composition serves to provide both early relief to the patient, and sustained relief over time. Sustained relief, by decreasing the number of

daily administrations required, is expected to increase patient compliance with a course of active agent treatments.

Solid or liquid particulate active agent prepared for practicing the present invention should as noted above include particles of respirable or non-respirable size: that is, for respirable particles, particles of a size sufficiently small to pass through the mouth and larynx upon inhalation and into the bronchi and alveoli of the lungs, and for nonrespirable particles, particles sufficiently large to be retained in the nasal airway passages rather than pass through the larynx and into the bronchi and alveoli of the lungs. In general, particles ranging from about 1 to 5 microns in size (more particularly, less than about 4.7 microns in size) are respirable. Particles of non-respirable size are greater than about 5 microns in size, up to the size of visible droplets. Thus, for nasal administration, a particle size in the range of 10-500 μm may be used to ensure retention in the nasal cavity.

The dosage of active compound will vary depending on the condition being treated and the state of the subject, but generally may be an amount sufficient to achieve dissolved concentrations of active compound on the nasal airway surfaces of the subject of from about 10^{-9} to about 10^{-3} Moles/liter, and more preferably from about 10^{-6} to about 3×10^{-4} Moles/liter. Depending upon the solubility of the particular formulation of active compound administered, the daily dose may be divided among one or several unit dose administrations. The daily dose by weight may range from about .1, .5 or 1 to 10 or 20 milligrams of active agent particles for a human subject, depending upon the age and condition of the subject. A currently preferred unit dose is about .5, 1 or 2 milligrams of active agent given at a regimen of four administrations per day. The dosage may be provided as a prepackaged unit by any suitable means (e.g., encapsulating in a gelatin capsule).

Pharmaceutical formulations suitable for nasal administration include formulations of solutions, emulsions, suspensions and extracts. *See generally*, J. Nairn, Solutions, Emulsions, Suspensions and Extracts, in *Remington: The Science and Practice of Pharmacy*, chap. 86 (19th ed 1995). Pharmaceutical formulations suitable for nasal administration may be prepared as described in U.S. Patents Nos. 4,389,393 to Schor; 5,707,644 to Illum; 4,294,829 to Suzuki; and 4,835,142 to Suzuki; the disclosures of which are incorporated by reference herein in their entirety.

In the manufacture of a formulation according to the invention, active agents or the physiologically acceptable salts or free bases thereof are typically admixed with, inter alia, an acceptable carrier. The carrier must, of course, be acceptable in the sense of being compatible with any other ingredients in the formulation and must not be deleterious to the patient. The carrier may be a solid or a liquid, or both, and is preferably formulated with the compound as a unit-dose formulation, for example, a capsule, which may contain from 0.5% to 99% by weight of the active compound. One or more active compounds may be incorporated in the formulations of the invention, which formulations may be prepared by any of the well-known techniques of pharmacy consisting essentially of admixing the components.

Mists or aerosols of liquid particles comprising the active compound may be produced by any suitable means, such as by a simple nasal spray with the active agent in an aqueous pharmaceutically acceptable carrier, such as sterile saline solution or sterile water. Administration may be with a pressure-driven aerosol nebulizer or an ultrasonic nebulizer. See, e.g., U.S. Pat. No. 4,501,729 and 5,656,256. Suitable formulations for use in a nasal droplet or spray bottle or in nebulizers consist of the active ingredient in a liquid carrier, the active ingredient comprising up to 40% w/w of the formulation, but preferably less than 20% w/w. The carrier is typically water (and most preferably sterile, pyrogen-free water) or a dilute aqueous alcoholic solution, preferably made isotonic with body fluids by the addition of, for example, sodium chloride. Optional additives include preservatives if the formulation is not made sterile, for example, methyl hydroxybenzoate, antioxidants, flavoring agents, volatile oils, buffering agents and surfactants.

Mists or aerosols of solid particles comprising the active compound may likewise be produced with any solid particulate medicament aerosol generator. Aerosol generators for administering solid particulate medicaments to a subject produce particles which are respirable or non-respirable, as explained above, and generate a volume of mist or aerosol containing a predetermined metered dose of a medicament at a rate suitable for human administration. One illustrative type of solid particulate aerosol generator is an insufflator. Suitable formulations for administration by insufflation include finely comminuted powders which may be delivered by means of an insufflator or taken into the nasal cavity in the manner of a snuff. In the insufflator, the powder (e.g., a metered dose thereof effective to carry out

the treatments described herein) is contained in capsules or cartridges, typically made of gelatin or plastic, which are either pierced or opened in situ and the powder delivered by air drawn through the device upon inhalation or by means of a manually-operated pump. The powder employed in the insufflator consists either solely of the active ingredient or of a powder blend comprising the active ingredient, a suitable powder diluent, such as lactose, and an optional surfactant. The active ingredient typically comprises from 0.1 to 100 w/w of the formulation. A second type of illustrative aerosol generator comprises a metered dose inhaler. Metered dose inhalers are pressurized aerosol dispensers, typically containing a suspension or solution formulation of the active ingredient in a liquified propellant. During use these devices discharge the formulation through a valve adapted to deliver a metered volume, typically from 10 to 150 μ l to produce a fine particle spray containing the active ingredient. Suitable propellants include certain chlorofluorocarbon compounds, for example, dichlorodifluoromethane, trichlorofluoromethane, dichlorotetrafluoroethane and mixtures thereof. The formulation may additionally contain one or more co-solvents, for example, ethanol, surfactants (such as oleic acid or sorbitan trioleate), antioxidants and suitable flavoring agents.

Compositions containing respirable or non-respirable dry particles of micronized active agent may be prepared by grinding the dry active agent with a mortar and pestle, and then passing the micronized composition through a 400 mesh screen to break up or separate out large agglomerates.

The particulate active agent composition may optionally contain a dispersant which serves to facilitate the formation of an aerosol. A suitable dispersant is lactose, which may be blended with the active agent in any suitable ratio (e.g., a 1 to 1 ratio by weight).

4. Covalent conjugates.

As noted above, also disclosed is a method of hydrating nasal airway surfaces in a subject in need of such treatment, comprising topically applying a sodium channel blocker to a nasal airway surface of the subject in an amount effective to inhibit the reabsorption of water by the nasal airway surface, wherein the sodium channel blocker is a covalent conjugate of a pyrazinoylguanidine sodium channel blocker and a non-absorbable carrier moiety. The non-absorbable carrier moiety may be a carbohydrate, protein, peptide, amino acid, polyamine, or water soluble linear polymer, which may be directly linked to the sodium channel blocker or linked through an intermediate linking group such as an alkylene group. Other suitable carrier moieties include erythritol and xylitol.

Water soluble linear polymers useful as carrier moieties include polyvinylpyrrolidone, polyethylene glycol, nonylphenol ethoxylates, and polyvinyl alcohol.

Carbohydrates useful as carrier moieties include sugars and polysaccharides (including charged and uncharged polysaccharides), such as dextran, lactose, sialic acid and mannitol. An additional example is agarose.

Proteins or peptides useful as carrier moieties include albumin (for example, human serum albumin) and protamine.

Amino acids useful for carrying out the present invention include all twenty standard amino acids (alanine, valine, leucine, isoleucine, proline, phenylalanine, tryptophan, methionine, glycine, serine, threonine, cysteine, tyrosine, asparagine, glutamine, aspartic acid, glutamic acid, lysine, arginine, and histidine), in either the D or L configuration.

Polyamines useful for carrying out the present invention include spermine and spermidine.

In one embodiment, the conjugate has the formula:

wherein:

X, Y, and R₂ are as described above, and Z is the non-absorbable carrier moiety covalently linked, directly or indirectly, to the adjacent nitrogen atom.

An of a variety of approaches for linking the pyrazinoylguanidine sodium channel blocker to the non-absorbable moiety may be employed, as discussed below.

A direct linkage by a caproate linker may be employed by reacting the nonabsorbable moiety with intermediates as follows, in accordance with known techniques:

5

where X, Y, and R₂ are as given above.

Spacer linkers may be employed with intermediates as follows:

(T. Kleyman and E. Cragoe, *J. Membrane Biol.* **105**, 1 (1988)(Fig. 2)

where X, Y, and R₂ are as given above.

10

General linkers may be employed with intermediates as follows:

where X and Y are as described above.

Techniques for forming conjugates are known. A covalent conjugate of amiloride and bovine or rabbit serum albumin for use in creating anti-amiloride antibodies is disclosed in T. Kleyman et al., *New Amiloride Analogue as Hapten to Raise Anti-Amiloride Antibodies*, *Am. J. Physiol.* **250**, C165-C170 (1986). This same technique can be performed with other amiloride analogs, and with other proteins or peptides. Thus, for example, a covalent conjugate of amiloride and analogs thereof, such as benzamil or phenamil, with human serum albumin can be produced in like manner.

20

Covalent conjugates of amiloride and SEPHAROSE™ brand agarose (a polysaccharide) are described in T. Kleyman et al., *supra*, and in D. Benos et al., *Proc. Natl. Acad. Sci. USA* **83**, 8525-8529 (1986). Different brands, forms, and molecular weights of agarose may be employed, or other polysaccharides employed, or different amiloride analogs such as benzamil or phenamil may be employed, to make additional covalent conjugates by similar techniques.

25

5. P2Y₂ Receptor agonists.

The methods described above may further comprise the step of topically applying a P2Y₂ receptor agonist to a nasal airway surface of the subject in an amount effective to stimulate chloride secretion by the nasal airway surface. The concurrent topical application may be carried out by combining the P2Y₂ receptor agonists with the active agent described above in a common pharmaceutically acceptable carrier

30

solution. The agonist may be UTP or an analog thereof, as described in U.S. Patents Nos. 5,789,391; 5,656,256 or 5,292,498, the disclosures of which are to be incorporated by reference herein in their entirety. Particularly preferred agonists are UTP, the UTP analog uridine 5'-O-(3-thiotriphosphate) (or "UTP gamma S"), or the
5 UTP analog P^1, P^4 -di (uridine-5') tetraphosphate (or " U_2P_4 ").

Concurrent application refers to essentially co-extensive application, or application to the same portion or portions of the nasal airway surfaces. Such concurrent or co-extensive application can be achieved by simultaneous administration, or by administration sufficiently close in time so that the two agents
10 achieve their therapeutic effect concurrently.

Additional examples of receptor agonists that can be used to carry out the present invention include those having the general formula:

15

20 wherein:

A and B are each independently a purine or pyrimidine base (e.g., adenine, guanine, thymine, cytosine, uracil); preferably, A is uracil and B is cytosine;

R_1 and R_2 are each independently selected from the group consisting of H or OH; and

25 n is from 1 to 5 or 6, preferably 2, 3 or 4.

The receptor agonists may be combined in the same formulation and administered in like manner as the active compounds described above, or as described in U.S. Patent No. 5,656,256 or 5,292,498, the disclosures of which are to be incorporated by reference herein in their entirety.

30 The foregoing is illustrative of the present invention, and is not to be construed as limiting thereof. The invention is defined by the following claims, with equivalents of the claims to be included therein.

What is Claimed is:

1. A method of hydrating nasal airway surfaces in a subject in need of such treatment, comprising:

5 topically applying a sodium channel blocker to a nasal airway surface of said subject in an amount effective to inhibit the reabsorption of water by said nasal airway surface.

2. A method according to claim 1, wherein said patient is receiving nasal oxygen supplementation.

10 3. A method according to claim 1, wherein said patient is afflicted with an allergic disorder affecting nasal airway surfaces.

4. A method according to claim 1, wherein said patient is afflicted with an inflammatory disorder affecting nasal airway surfaces.

5. A method according to claim 1, wherein said patient is afflicted with a bacterial infection of nasal airway surfaces.

20 6. A method according to claim 1, wherein said sodium channel blocker is a pyrazinoylguanidine sodium channel blocker.

7. A method according to claim 6, wherein said pyrazinoylguanidine sodium channel blocker has the formula:

25 wherein:

X is selected from the group consisting of chloro, bromo, iodo, loweralkyl, lower-cycloalkyl having from 3 to 7 carbons, phenyl, chlorophenyl, bromophenyl, Z-thio and Z-sulfonyl wherein Z is selected from the group consisting of loweralkyl and phenyl-loweralkyl;

30 Y is selected from the group consisting of hydroxyl, mercapto, loweralkyloxy, loweralkylthio, chloro, loweralkyl, lowercycloalkyl having from 3 to 6 carbons, phenyl, amino having the structure:

wherein

R is selected from the group consisting of hydrogen, amino, amidino, lower-cycloalkyl having 3 to 6 carbon atoms, loweralkyl, hydroxyloweralkyl, halo-loweralkyl, lower-(cycloalkylalkyl) having 3 to 6 carbons in the ring, phenyl-loweralkyl, lower-(alkylaminoalkyl), lower-alkenyl, phenyl, halophenyl, and lower-alkylphenyl;

R₁ is selected from the group consisting of hydrogen, loweralkyl, loweralkenyl, and additionally;

R and R₁ can be joined to form a lower alkylene;

R₂ is selected from the group consisting of hydrogen and loweralkyl; and

R₃ and R₄ are independently selected from the group consisting of hydrogen, loweralkyl, hydroxy-loweralkyl, phenyl-loweralkyl, (halophenyl)-loweralkyl, lower-(alkylphenylalkyl), (loweralkoxyphenyl)-loweralkyl, naphthyl-loweralkyl, (octahydro-1-azocinyl)-loweralkyl, pyridyl-loweralkyl, and loweralkyl radicals linked to produce with the nitrogen atom to which they are attached a 1-pyrrolidinyl, piperidino, morpholino, and a 4-loweralkyl-piperazinyl group, and phenyl; or a pharmaceutically acceptable salt thereof.

8. A method according to claim 1, wherein X is chloro.

9. A method according to claim 1, wherein Y is amino.

10. A method according to claim 1, wherein R, R₁, and R₂ are hydrogen.

11. A method according to claim 1, wherein R₃ is hydrogen, phenyl, or phenylalkyl.

12. A method according to claim 1, wherein R₄ is hydrogen.

13. A method according to claim 1, further comprising the step of topically applying a P2Y₂ receptor agonist to a nasal airway surface of said subject in an amount effective to stimulate chloride secretion by said nasal airway surface.

14. A method of hydrating a mucosal surface in a subject in need of such treatment, comprising:

topically applying a sodium channel blocker to a nasal airway surface of said subject in an amount effective to inhibit the reabsorption of water by said mucosal surface,

wherein said sodium channel blocker is a covalent conjugate of a pyrazinoylguanidine sodium channel blocker and a non-absorbable carrier moiety.

15. A method according to claim 14, wherein said mucosal surface is a nasal airway surface and said patient is receiving nasal oxygen supplementation.

16. A method according to claim 14, wherein said mucosal surface is a nasal airway surface and said patient is afflicted with an allergic disorder affecting nasal airway surfaces.

17. A method according to claim 14, wherein said mucosal surface is a nasal airway surface and said patient is afflicted with an inflammatory disorder affecting nasal airway surfaces or a bacterial infection of the nasal airway surfaces.

18. A method according to claim 14, wherein said non-absorbable carrier moiety is selected from the group consisting of carbohydrates, proteins, peptides, polyamines, and water soluble linear polymers.

19. A method according to claim 18, wherein said non-absorbable carrier moiety is a water soluble linear polymer selected from the group consisting of polyvinylpyrrolidone, polyethylene glycol, nonylphenol ethoxylates, polyvinyl alcohol.

20. A method according to claim 18, wherein said non-absorbable carrier moiety is a carbohydrate selected from the group consisting of sugars and polysaccharides.

21. A method according to claim 18, wherein said non-absorbable carrier moiety is a carbohydrate selected from the group consisting of dextran, lactose, sialic acid, and mannitol.

5 22. A method according to claim 18, wherein said non-absorbable carrier moiety is a protein or peptide selected from the group consisting of albumin and protamine.

10 23. A method according to claim 18, wherein said non-absorbable carrier moiety is a polyamine selected from the group consisting of spermine and spermidine.

24. A method according to claim 14, wherein said conjugate has the formula:

15 wherein:

X is selected from the group consisting of chloro, bromo, iodo, loweralkyl, lower-cycloalkyl having from 3 to 7 carbons, phenyl, chlorophenyl, bromophenyl, X'-thio and X'-sulfonyl wherein X' is selected from the group consisting of loweralkyl and phenyl-loweralkyl;

20 Y is selected from the group consisting of hydroxyl, mercapto, loweralkyloxy, loweralkylthio, chloro, loweralkyl, lowercycloalkyl having from 3 to 6 carbons, phenyl, amino having the structure:
wherein

25 R is selected from the group consisting of hydrogen, amino, amidino, lower-cycloalkyl having 3 to 6 carbon atoms, loweralkyl, hydroxyloweralkyl, halo-loweralkyl, lower-(cycloalkylalkyl) having 3 to 6 carbons in the ring, phenyl-loweralkyl, lower-(alkylaminoalkyl), lower-alkenyl, phenyl, halophenyl, and lower-alkylphenyl;

30 R₁ is selected from the group consisting of hydrogen, loweralkyl, loweralkenyl, and additionally;

R and R₁ can be joined to form a lower alkylene;

R₂ is selected from the group consisting of hydrogen and loweralkyl; and

Z is a non-absorbable carrier moiety covalently linked to the adjacent nitrogen atom;

or a pharmaceutically acceptable salt thereof.

5 25. A method according to claim 24, wherein X is chloro and Y is amino.

26. A method according to claim 24, wherein X is chloro and Y is chloro.

10 27. A method according to claim 24, wherein R, R₁, and R₂ are hydrogen.

28. A method according to claim 14, further comprising the step of topically applying a P2Y₂ receptor agonist to a mucosal surface of said subject in an amount effective to stimulate chloride secretion by said mucosal surface.

15 29. A pharmaceutical formulation, comprising:
a sodium channel blocker in a pharmaceutically acceptable carrier;
wherein said sodium channel blocker is a covalent conjugate of a
pyrazinoylguanidine sodium channel blocker and a non-absorbable carrier moiety;
subject to the proviso that said non-absorbable carrier moiety is not bovine
20 serum albumin or rabbit serum albumin.

30. A pharmaceutical formulation according to claim 29, said sodium channel blocker included in said composition in an amount effective to inhibit the reabsorption of water by mucosal surfaces.

25 31. A pharmaceutical formulation according to claim 29, said sodium channel blocker included in said composition in an amount effective to inhibit the reabsorption of water by mucosal surfaces.

30 32. A pharmaceutical formulation according to claim 29, wherein said non-absorbable carrier moiety is selected from the group consisting of carbohydrates, proteins, peptides, polyamines, and water soluble linear polymers.

33. A pharmaceutical formulation according to claim 29, wherein said non-absorbable carrier moiety is a water soluble linear polymer selected from the group consisting of polyvinylpyrrolidone, polyethylene glycol, nonylphenol ethoxylates, and polyvinyl alcohol.

5

34. A pharmaceutical formulation according to claim 29, wherein said non-absorbable carrier moiety is a carbohydrate selected from the group consisting of sugars and polysaccharides.

10

35. A pharmaceutical formulation according to claim 29, wherein said non-absorbable carrier moiety is a carbohydrate selected from the group consisting of dextran, lactose, sialic acid, and mannitol.

15

36. A pharmaceutical formulation according to claim 29, wherein said non-absorbable carrier moiety is a protein or peptide selected from the group consisting of albumin and protamine.

20

37. A pharmaceutical formulation according to claim 29, wherein said non-absorbable carrier moiety is a polyamine selected from the group consisting of spermine and spermidine.

38. A pharmaceutical formulation according to claim 29, wherein said conjugate has the formula:

25 wherein:

X is selected from the group consisting of chloro, bromo, iodo, loweralkyl, lower-cycloalkyl having from 3 to 7 carbons, phenyl, chlorophenyl, bromophenyl, X'-thio and X'-sulfonyl wherein X' is selected from the group consisting of loweralkyl and phenyl-loweralkyl;

30

Y is selected from the group consisting of hydroxyl, mercapto, loweralkyloxy, loweralkylthio, chloro, loweralkyl, lowercycloalkyl having from 3 to 6 carbons, phenyl, amino having the structure:
wherein

R is selected from the group consisting of hydrogen, amino, amidino, lower-cycloalkyl having 3 to 6 carbon atoms, loweralkyl, hydroxyloweralkyl, halo-loweralkyl, lower-(cycloalkylalkyl) having 3 to 6 carbons in the ring, phenyl-loweralkyl, lower-(alkylaminoalkyl), lower-alkenyl, phenyl, halophenyl, and lower-alkylphenyl;

R₁ is selected from the group consisting of hydrogen, loweralkyl, loweralkenyl, and additionally;

R and R₁ can be joined to form a lower alkylene;

R₂ is selected from the group consisting of hydrogen and loweralkyl; and

Z is a non-absorbable carrier moiety covalently linked to the adjacent nitrogen atom;

or a pharmaceutically acceptable salt thereof.

39. A pharmaceutical formulation according to claim 38, wherein X is chloro and Y is chloro.

40. A pharmaceutical formulation according to claim 38, wherein X is chloro and Y is amino.

41. A pharmaceutical formulation according to claim 38, wherein R, R₁, and R₂ are hydrogen.

42. A pharmaceutical formulation according to claim 29, further comprising a P2Y₂ receptor agonist.

43. A pharmaceutical formulation according to claim 42, said P2Y₂ receptor agonist included in an amount effective to stimulate chloride secretion by airway surfaces.

44. A pharmaceutical formulation according to claim 42, said P2Y₂ receptor agonist included in an amount effective to stimulate chloride secretion and water secretion by airway surfaces.

45. A pharmaceutical formulation according to claim 29, wherein said carrier is an aqueous carrier solution.

46. A compound useful as a sodium channel blocker, said compound comprising a covalent conjugate of a pyrazinoylguanidine sodium channel blocker and a non-absorbable carrier moiety;

subject to the proviso that said non-absorbable carrier moiety is not bovine serum albumin, rabbit serum albumin or agarose.

47. A compound according to claim 46, wherein said non-absorbable carrier moiety is selected from the group consisting of carbohydrates, proteins, peptides, polyamines, and water soluble linear polymers.

48. A compound according to claim 46, wherein said non-absorbable carrier moiety is a water soluble linear polymer selected from the group consisting of polyvinylpyrrolidone, polyethylene glycol, nonylphenol ethoxylates, polyvinyl alcohol.

49. A compound according to claim 46, wherein said non-absorbable carrier moiety is a carbohydrate selected from the group consisting of sugars and polysaccharides.

50. A compound according to claim 46, wherein said non-absorbable carrier moiety is a carbohydrate selected from the group consisting of dextran, lactose, sialic acid and mannitol.

51. A compound according to claim 46, wherein said non-absorbable carrier moiety is a protein or peptide selected from the group consisting of albumin and protamine.

52. A compound according to claim 46, wherein said non-absorbable carrier moiety is a polyamine selected from the group consisting of spermine and spermidine.

53. A compound according to claim 46, wherein said conjugate has the formula:

wherein:

5 X is selected from the group consisting of chloro, bromo, iodo, loweralkyl, lower-cycloalkyl having from 3 to 7 carbons, phenyl, chlorophenyl, bromophenyl, X'-thio and X'-sulfonyl wherein X' is selected from the group consisting of loweralkyl and phenyl-loweralkyl;

Y is selected from the group consisting of hydroxyl, mercapto, loweralkyloxy, loweralkylthio, chloro, loweralkyl, lowercycloalkyl having from 3 to 6 carbons, phenyl, amino having the structure:

wherein

R is selected from the group consisting of hydrogen, amino, amidino, lower-cycloalkyl having 3 to 6 carbon atoms, loweralkyl, hydroxyloweralkyl, halo-loweralkyl, lower-(cycloalkylalkyl) having 3 to 6 carbons in the ring, phenyl-loweralkyl, lower-(alkylaminoalkyl), lower-alkenyl, phenyl, halophenyl, and lower-alkylphenyl;

R₁ is selected from the group consisting of hydrogen, loweralkyl, loweralkenyl, and additionally;

20 R and R₁ can be joined to form a lower alkylene;

R₂ is selected from the group consisting of hydrogen and loweralkyl; and

Z is a non-absorbable carrier moiety covalently linked to the adjacent nitrogen atom;

or a pharmaceutically acceptable salt thereof.

25

54. A compound according to claim 53, wherein X is chloro and Y is chloro.

55. A compound according to claim 53, wherein X is chloro and Y is amino.

30 56. A compound according to claim 53, wherein R, R₁, and R₂ are hydrogen.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US99/24546

A. CLASSIFICATION OF SUBJECT MATTER

IPC(7) : A61F 13/00

US CL : Please See Extra Sheet.

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 424/434

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

BRS, CAS Online, Medline

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X - Y	US 5,656,256 A (BOUCHER et al.) 12 August 1997, see entire document.	1, 6, 13 ---- 1-6, 13-21, 28-32, 34-37, 42-47, 49- 50
X,P --- Y,P	US 5,876,700 A (BOUCHER, Jr. et al.) 02 March 1999 see entire document	1, 6, 13 ---- 1-6, 13-21, 28-32, 34-37, 42-47, 49- 50

☒ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

* *A* *B* *L* *O* *P*	Special categories of cited documents: document defining the general state of the art which is not considered to be of particular relevance earlier document published on or after the international filing date document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified) document referring to an oral disclosure, use, exhibition or other means document published prior to the international filing date but later than the priority date claimed	*T* *X* *Y* *a*	later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art document member of the same patent family
--------------------------------------	---	--------------------------	--

Date of the actual completion of the international search

02 FEBRUARY 2000

Date of mailing of the international search report

29 FEB 2000

Name and mailing address of the ISA/US
Commissioner of Patents and Trademarks
Box PCT
Washington, D.C. 20231

Facsimile No. (703) 305-3230

Authorized officer

TODD D WARE

Telephone No. (703) 308-1234

Form PCT/ISA/210 (second sheet)(July 1992)*

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US99/24546

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X - Y	KNOWLES, M.R. et al. Activation by extracellular nucleotides of chloride secretion in the airway epithelia of patients with cystic fibrosis. New Eng. J. Med. August 1991, Vol. 325, No. 8, pages 533-538, esp. 536-538.	1, 6, 13 ---- 1-6, 13
X - Y	US 5,635,160 A (STUTTS, III et al.) 03 June 1997 see entire document.	1, 6, 13 ---- 1-6, 13-21, 28-32, 34-37, 42-47, 49- 50

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US99/24546

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This international report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☒ Claims Nos.: 7-12, 24-28, 38-41, 53-56
because they relate to subject matter not required to be searched by this Authority, namely:

These claims either lack or are dependent from a claim lacking, but in need thereof, a chemical structural formula designating what X, Y, Z, R, R1-R3 are. It appears that a formula was intended to be submitted, but was mistakenly omitted. Accordingly, these claims were unsearchable.
2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:
3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:
4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

- ☐ The additional search fees were accompanied by the applicant's protest.
☐ No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US99/24546

A. CLASSIFICATION OF SUBJECT MATTER:

US CL :

424/434